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Chiral recognition of bilirubin and biliverdin in liposomes and micelles

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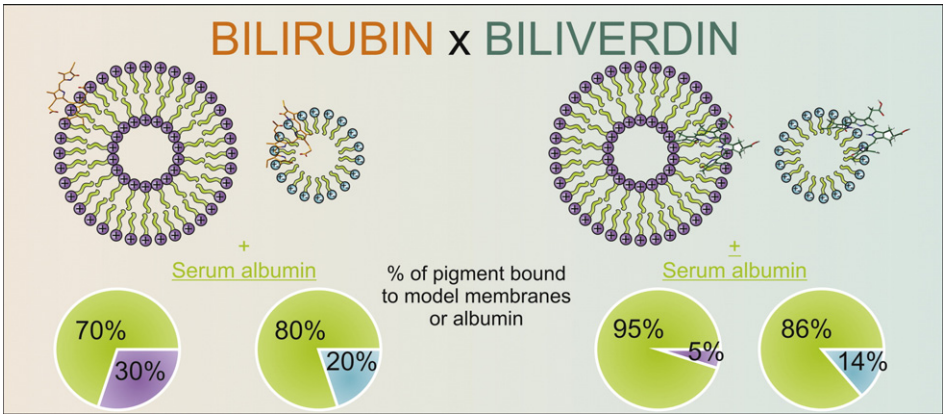
HIGHLIGHTS

- We studied the interaction of bilirubin and biliverdin with model membranes.
- A differing interaction with liposomes and micelles was observed for the pigments.
- The different nature of the pigments influenced their penetration into the bilayer.
- In the presence of liposomes, bilirubin favorably interacted with serum albumin.
- The presence of surfactant completely changed biliverdin binding to the protein.

GRAPHICAL ABSTRACT



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ABSTRACT

The structural formula of biologically important chiral pigments bilirubin and biliverdin differs only by one double bond. We showed that this results in dissimilar interactions with two models of membranes: cationic liposomes composed of 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl]cholesterol and zwitterionic micelles from 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). While the liposomes recognized the P-form of bilirubin, the micelles recognized its M-form. Both recognized the P-form of biliverdin.
Our study also comprised ternary systems consisting of the pigments, model membranes and serum albumin (human and bovine). Bilirubin preferentially interacted with the albumins even in the presence of the liposomes. On the other hand, biliverdin preferred the liposomes. Remarkably, the presence of CHAPS completely changed the biliverdin binding to the protein.
Because our study was oriented on different chiral interactions, a chiroptical method of electronic circular dichroism was chosen as the principal method to study our systems. As complementary methods, UV–vis absorption and fluorescence emission were used.

Abbreviations: BR, bilirubin; BV, biliverdin; BSA, bovine serum albumin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CMC, critical micellar concentration; DC-cholesterol, 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl]cholesterol hydrochloride; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; ECD, electronic circular dichroism; HSA, human serum albumin; IR, infrared; UV–vis, ultraviolet–visible.

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1. Introduction

Our study is focused on two naturally occurring bile pigments: yellow-orange bilirubin (BR) and green-blue biliverdin (BV). BV is formed as a result of an oxidative breakdown of the heme moiety of hemoglobin and is usually immediately reduced to free BR. However, it can be for instance seen as a cause of a green color in bruises. On the other hand, BR is standardly occurring in low concentrations in blood plasma. Both of the pigments may be found in an excess in the blood during the hepatic diseases like jaundice [1,2].

BR and BV are linear tetrapyrroles and their structural formula differs only by one double bond localized in the central part of the BV molecule (Fig. 1). However, their spatial structure differs substantially [3–6]. In solution, BR is normally stabilized by six intramolecular hydrogen bonds while the double bond in BV restricts its possible conformations and, hence, there are only two intramolecular hydrogen bonds in BV. The spatial structure gives rise to two enantiomeric forms of both bile pigments: the P- and M-helical forms (Fig. 1). The racemization barrier between the two enantiomers is, nevertheless, very low and therefore we always get a racemate in a solution without a chiral selecting agent.

In the presence of a chiral selector, BR and BV often preferably interact only in one of their enantiomeric forms forming one diastereoisomeric complex with the selector [7–16]. Selectors of different chemical natures have been used previously and described in the literature: proteins and peptides [7,10,11,17–21], cyclodextrins [8,13–16], metals [9], alkaloids [22] and guanosine assemblies [23] among others. A considerable part of the studies [21,24–29] is devoted to BR interactions with different models of membranes — either liposomal or micellar. These studies were inspired partly by a search for a good chiral selector of BR, also by describing the chiral properties of the models and last but not the least by the biological effects of increased BR concentrations. The studies proved the enantioselective interaction of BR with models of membranes — either the P- or M-form of BR was preferentially recognized by the membranes. The enantioselective attack of BR on the nerve cells is one of the most debated negative effects of this pigment in our organism. Therefore different models of membranes were previously studied to explain these properties of BR.

Our work focuses on two non-standard models of membranes and their interaction with both BR and BV. We studied cationic liposomes composed of chiral 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl]cholesterol (DC-cholesterol, structure in Fig. 2). The interaction of bile pigments with positively charged model membranes has been scarcely studied before for BR [30] and never for BV. Interestingly, the BV interactions with model membranes or with cellular membranes have not been described at all. We have chosen this specific model because of its charge and also because the main functional unit in its structure is very close to cholesterol. Previously, we have studied [21] BR interactions with different zwitterionic and negatively charged liposomes and also with their mixtures with cholesterol, but we did not observe any specific interaction with cholesterol. Our unpublished results also showed that BV did not interact enantioselectively with classical membrane models from zwitterionic 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and sphingomyelin to negatively charged 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine liposomes.

The newly used cationic model should simulate the positively charged parts of cellular membrane surfaces. However, the DC-cholesterol liposomes could also serve as an interesting chiral selecting agent for both pigments if the liposomes were to interact only with one of the enantiomeric forms of the pigments.

As a comparative model, micelles from zwitterionic 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, structure in Fig. 2) were chosen. This compound also has a functional group consisting of several cycles, in this case of an aliphatic nature. We suppose that these micelles can be used as a good and relatively simple selector for the enantioselective recognition of BR and BV.

Our study also includes ternary systems consisting of bile pigments, model membranes and serum albumin. We included serum albumin in our study because BR and BV are both known to bind to this protein enantioselectively [7,11,17–20] and this interaction is vital as serum albumin works as the main transporter of BR in the blood circulation [31] and it plays an important role during the BR and BV oxidative cycles [31]. We primarily chose human serum albumin (HSA) as studies with this protein are of human health importance. HSA has three binding sites with P-selectivity of BR: one primary site with a considerably high binding constant ($K_a \sim 10^8\ M^{-1}$) and two secondary sites with lower and similar binding constants ($K_a \sim 10^6\ M^{-1}$) [18–20,32–34]. HSA has also at least two binding sites for BV with the M-stereoselectivity [35–37]. Therefore, it is interesting to follow such ternary systems, especially to clarify whether bile pigments would prefer to bind to HSA or to the model membranes establishing their potential toxicity towards cell membranes with a positive charge.

As a comparative protein, especially for the study with BR, bovine serum albumin (BSA) was chosen. BSA has an opposite stereoselectivity of the bilirubin primary binding site as compared with HSA but the two secondary binding sites also have P-stereoselectivity [7,17]. However, it has an M-stereoselectivity for BV similar to that of HSA and, moreover,

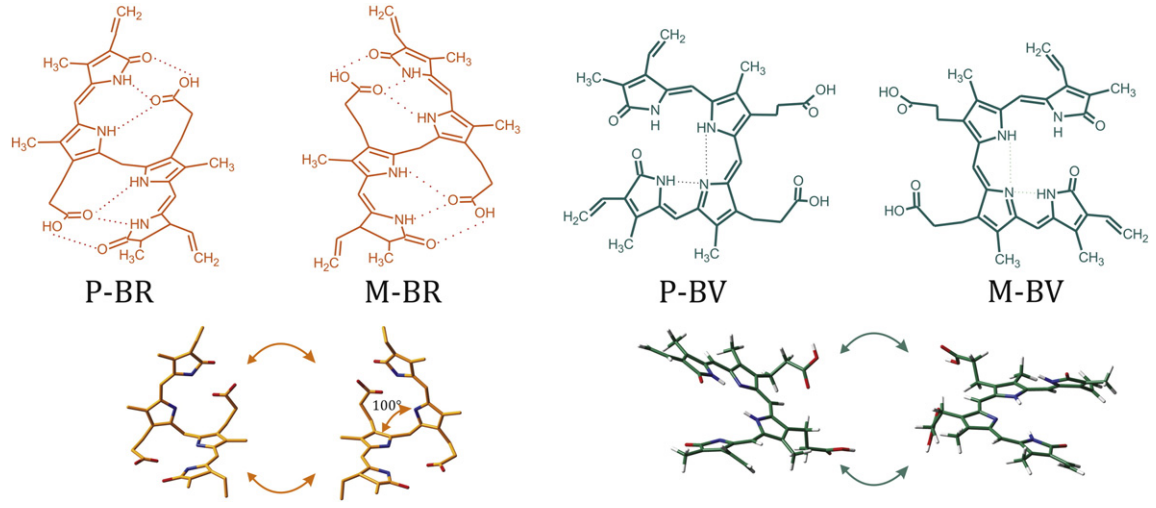

Fig. 1. The structural formulas of the enantiomeric P- and M-helical forms of BR and BV and their spatial chiral structures, denoted as P-BR, M-BR and P-BV, M-BV. The hydrogen bonds are shown as dotted lines.